

Development and Emergence of the Alfalfa Pollinator *Megachile rotundata* (Hymenoptera: Megachilidae)

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ABSTRACT *Megachile rotundata* (F.), a gregarious, cavity-nesting, leaf-cutting bee, is used throughout North America for the pollination of alfalfa, *Medicago sativa* L., seed crops. We examined the influence of various temperature regimes on development, survival, emergence, and longevity in both nondiapausing and diapausing forms of this species. In general, development rates increased with increasing constant temperatures used in this study (18, 22, 26, and 29°C), but the 26 and 29°C treatments were clearly superior as rearing temperatures for immatures. In diapausing individuals, a variable temperature treatment 14:27°C (8:16 h daily cycle, mean 22°C) reduced the length of prepupal and pupal development stages following incubation in the early summer when compared with individuals reared under the constant 22°C treatment. We discuss the importance of differing temperature regimes on *M. rotundata* development, survival, and longevity over the entire life cycle. We also discuss the importance of making a connection between immature development and sufficient wintering conditions to postdiapause development, a topic that has received much more attention in the literature.

KEY WORDS alfalfa leafcutting bee, development, wintering, emergence, mortality, pollination

THE ALFALFA LEAF-CUTTING bee, *Megachile rotundata* (F.), is a gregarious, cavity-nesting, leaf-cutting bee native to southwestern Asia and southeastern Europe. The alfalfa leafcutting bee was introduced to North America, perhaps several times beginning in the 1930s, and has had an interesting pattern of establishment and ultimately commercialization (Bohart 1970, 1972; Gruszka 1982). Currently, the alfalfa leafcutting bee, the pollinator of choice for alfalfa, *Medicago sativa* L., seed on >70,000 ha throughout western North America, is the most widely used commercially managed pollinator, after the honey bee, *Apis mellifera* L. In 1990 dollars, more than \$11 million is spent annually on alfalfa leafcutting bees in the United States alone (Peterson et al. 1992).

After emergence and mating during June and July each year, at most North American latitudes, female alfalfa leafcutting bees start building their nests in preexisting cavities such as beetle burrows in trees along river courses, cavities associated with farm buildings, or artificial nesting materials provided by alfalfa seed producers (Stephen 1981, Rank and Goerzen 1982, Richards 1984). Nests consist of a linear series of cells delimited by cut-leaf partitions. Each cell is provisioned with a mass of pollen and nectar, on top of which an egg is deposited. Completed nests are sealed with a cut-leaf plug. *M. rotundata* is a polylectic species, but females are strongly attracted to flowers of the legume genera *Medicago* and *Melilotus*.

Because of its superiority as an alfalfa pollinator, methods to manage *M. rotundata* populations have received considerable attention (Bohart 1970, 1972;

Hobbs 1973; Stephen 1981; Richards 1984). Nesting shelters with artificial nesting materials and emerging bees are placed in alfalfa seed fields shortly after bloom initiation in June and July and removed after about 6 wk. Bee progeny obtained in artificial nesting materials are stored for the remainder of the year. By late summer, fifth-instar alfalfa leafcutting bees complete consumption of the pollen-nectar provision, defecate, and spin a cocoon with silk-like strands. In this stage (prepupa), most bees in a given population undergo a diapause period that lasts through the winter months, and under natural conditions complete their development through the adult stage and emergence as ambient temperatures increase during the following spring and early summer. Under artificial commercial rearing conditions, prepupae that have received a sufficient wintering period are incubated during the early spring to ensure the proper timing of pollinators with the characteristic flush of bloom in alfalfa seed fields each year (Stephen 1981, Rank and Goerzen 1982, Richards 1984). However, at most latitudes in North America, a small proportion of bees in a given population, especially from the earliest nests produced each year, will avert the late summer prepupal diapause and complete development during the current year (Kronic 1972, Johansen and Eves 1973, Bitner 1976, Hobbs and Richards 1976, Richards 1984). This interesting phenomenon commonly referred to as "second generation" has received some attention, but a clear explanation of how diapause is mediated in this species has yet to emerge (Taséi and Masure 1978,

Klostermeyer 1982, Parker and Tepedino 1982, Tepedino and Parker 1986, Rank and Rank 1989).

Although some aspects of the biology and management of *M. rotundata* have received considerable attention, few published accounts exist on the effect of temperature on egg to adult development of both diapausing and nondiapausing forms, and emergence and subsequent longevity (Taséi and Masure 1978, Undurraga 1978, Whitfield and Richards 1992). Because of the need to time bee emergence with alfalfa bloom, most of the research conducted on temperature-dependent development in *M. rotundata* has been directed toward a better understanding of appropriate wintering procedures, postdiapause development, and springtime emergence patterns (Stephen and Osgood 1965, Kronic and Hinks 1972, Rank and Goerzen 1982, Richards et al. 1987, Richards and Whitfield 1988, Murrell 1991, Peterson et al. 1992). Improved understanding of the effects of temperature on prediapause development is important to effectively manage *M. rotundata* populations for alfalfa seed pollination. Rearing temperature not only influences developmental mortality and ultimately adult vigor, but also determines emergence time (Bosch and Kemp 2000), and, therefore, synchronization with local alfalfa seed field bloom.

During 1998–1999, we studied the development, mortality, and emergence of *M. rotundata* under various laboratory temperature regimes and outside conditions. Our three principal objectives were as follows: (1) to identify treatments that were adequate for rearing both nondiapausing and diapausing forms within a *M. rotundata* population, (2) to compare our rearing temperature and emergence time results with previous research where available, and (3) to examine the connection between rearing temperatures and the improved management of vigorous emerging adult bees for alfalfa seed pollination.

Materials and Methods

Bees were obtained from an actively nesting population managed at our laboratory and released at the beginning of June 1998 in pasture containing alfalfa near Clarkston, UT (112.0265° W, 41.9135° N). Wooden boxes with polystyrene wafers and inserted paper straws (11.5 cm long, 5.5 mm diameter) were used as nesting materials. During peak nesting, a sample of newly plugged paper straws was removed from the polystyrene wafers each day and taken to the Bee Biology & Systematics Laboratory (BBSL), where the straws were dissected. Within each nest, cells with unhatched eggs were dated, assuming an approximate cell production rate of two cells per day (Klostermeyer et al. 1973, Klostermeyer 1982). Cells with hatched eggs were discarded. *M. rotundata* females are ≈ 1.2 times larger than males, and female eggs are allocated larger pollen-nectar provisions, which tend to be deposited in the innermost cells of nesting cavities (Klostermeyer et al. 1973, Klostermeyer 1982). Using these criteria, the first two cells within each straw were considered females, and the last two cells

males. The remaining cells were not used. Nests with fewer than five cells also were not used. Bee sex was confirmed in later developmental stages (pupae and adults). Provisions with eggs were transferred to artificial clay wells (Torchio and Bosch 1992, Bosch and Kemp 2000), which were labeled with nest number and cell position within the nest and covered with glass slide covers.

Male and female cells were assigned in equal numbers to various temperature treatments, so that no treatment received two cells from the same nest and sex. Sample sizes for laboratory treatments each ranged between 140 and 150 individuals, in roughly equal numbers of males and females. Clay wells with eggs and provisions were placed in clear polyvinyl chloride boxes containing two additional clay wells filled with water to provide adequate humidity throughout development. Boxes were transferred to temperature cabinets according to the following treatments: constant 18, 22, 26, 29°C, and variable 14:27°C on a thermoperiod of 8:16 h (mean: 22°C). Clay blocks were checked daily and the dates of hatching, beginning of defecation, beginning of cocoon spinning, and cocoon completion were noted. After cocoon completion, cocoons were placed individually in clear gel capsules and transferred to sticky boards (20 by 25 cm boards with double-sided adhesive tape), which were X-rayed every 3 d (Stephen and Undurraga 1976). X-ray plates were used to record the dates when bees pupated and became adults. Nondiapausing bees, bees that pupated in 1998, were allowed to remain in their originally assigned temperature treatment, and were checked daily for the adult development stage, as well as for adult emergence dates, whereupon they were removed from the gel capsules, individually transferred to a glass vial, incubated at 22°C, and monitored daily until death. Adult longevity without feeding was used as a measure of vigor (Bosch and Kemp 2000). In the case of diapausing bees in each of the laboratory treatments, after exposure to ramped reductions in temperatures with time at constant 18, 14, and 10°C during the prior week (to reduce the likelihood of thermal shock), prepupae were transferred to 4°C on 13 October 1998. Diapausing bees were held at 4°C for 203 d, after which they were taken out of the cooler on 4 May 1999, incubated under the respective conditions of their previously assigned treatments, and allowed to continue development and emerge. After emergence, bees were treated in the same way as nondiapausing bees during the previous autumn (1998).

One additional treatment (outside) was conducted to imitate naturally variable thermal conditions of Cache Valley, UT, during 1998–1999. Whole nests containing ≈ 365 individual *M. rotundata* were held in a small ventilated weather instrument shelter at the BBSL. Daily maximum, mean, and minimum temperatures were obtained from local NOAA weather reporting stations. Development (cocoon spinning, pupation, and adulthood) in these nests was monitored via X-ray plates taken every 3 d. Upon reaching adulthood, individual cells were dissected from the nests,

Table 1. Duration (in days) of developmental periods for nondiapausing male and female *M. rotundata* reared under differing temperature regimes (mean \pm SE)

Treatment, °C	Sex	Egg	Instar I-IV	Def./Spin.	Spin./Pupa	Pupa/Adult	Egg/Adult
18 ^a	♂						
	♀						
22	♂	3.4 \pm 0.50	8.9 \pm 0.29	3.1 \pm 0.27	17.4 \pm 0.36	25.9 \pm 0.52	58.7 \pm 1.08
	♀	3.5 \pm 0.24	8.0 \pm 0.41	2.7 \pm 0.36	18.8 \pm 0.56	29.5 \pm 0.64	62.5 \pm 0.89
26	♂	2.0 \pm 0.20	4.7 \pm 0.13	1.9 \pm 0.13	9.3 \pm 0.46	12.2 \pm 0.56	30.1 \pm 0.32
	♀	3.2 \pm 0.25	4.9 \pm 0.14	2.1 \pm 0.14	9.8 \pm 0.73	13.6 \pm 0.73	33.6 \pm 0.63
29	♂	2.1 \pm 0.13	3.9 \pm 0.11	1.7 \pm 0.16	8.8 \pm 0.64	8.0 \pm 0.64	24.2 \pm 0.34
	♀	3.1 \pm 0.28	4.3 \pm 0.24	1.4 \pm 0.20	9.3 \pm 0.73	10.1 \pm 0.55	28.2 \pm 0.56
14:27	♂	2.9 \pm 0.19	6.4 \pm 0.16	2.6 \pm 0.17	12.3 \pm 0.37	15.2 \pm 0.48	39.3 \pm 0.39
	♀	3.3 \pm 0.30	6.1 \pm 0.32	2.2 \pm 0.14	13.3 \pm 0.61	18.4 \pm 0.50	43.3 \pm 0.74
Outside	♂	—	—	—	9.3 \pm 0.28	14.5 \pm 0.29	32.4 \pm 0.37
	♀	—	—	—	11.3 \pm 0.35	16.5 \pm 0.36	36.9 \pm 0.53

Def./Spin., period from first observation of individuals defecating (initiation of fifth instar) to time when first strands of silk are produced for cocooning by fifth instar individuals. Spin./Pupa, period from first strands of silk produced by fifth instar individuals to prepare cocoon until molt to pupal stage within completed cocoon.

^a No individuals completed development and emerged during period of investigation.

transferred to clear gel capsules, and placed on sticky boards as described above for the other treatments. Sticky boards housed in the outside shelter were monitored daily for bee emergence. Upon emergence, bees were individually transferred to glass vials at 22°C and longevity was measured as described above.

We used *t*-test and analysis of variance procedures as appropriate to compare differences among treatments, bee type (nondiapausing and diapausing bees), and sex, in selected development periods—for example, total development time (days to develop from egg to adult), emergence time (days to emerge after incubation), and longevity (days from emergence until death at 22°C). Differences in developmental and winter mortality rates were analyzed with chi-square tests.

Results

The first nests were recovered from the Clarkston, UT, field site during the first week of July 1998. By 1 August, nearly all bees from the outside treatment had reached fifth instar and were spinning cocoons. Non-

diapausing larvae from the outside treatment were all pupae by 15 August, and adults by 29 August, and had emerged by 7 September. Nondiapausing male bees from the outside treatment required just over 1 mo to transit from egg to adult (Table 1), and developed significantly faster than females ($t = -6.97$, $df = 91$, $P < 0.01$). Weather station temperatures during the developmental period 1 July–10 September 1998 ranged from 19 to 29°C, with a mean of 23°C.

None of the bees reared under the 18°C treatment were able to complete development and emerge during the period of this investigation. No pupae from the 18°C treatment were observed during the autumn of 1998, and by 5 September 1999 only two males and four females had developed to the adult stage. For both nondiapausing and diapausing forms, increasing temperatures from 22 to 29°C reduced larval development times by $\approx 50\%$ and pupal development times by $\approx 60\%$ (Tables 1 and 2; Figs. 1 and 2). There were also significant reductions in total development time (egg to adult) with increasing temperatures for both nondiapausing ($F = 735.1$, $df = 4$, $P < 0.0001$) and diapausing

Table 2. Duration (in days) of development periods for diapausing male and female *M. rotundata* reared under differing temperature regimes (mean \pm SE)

Treatment, °C	Sex	Egg	Instar I-IV	Def./Spin.	Spin./Cool	Cool/Inc.	Inc./Pupa	Pupa/Adult	Egg/Adult
18 ^a	♂								
	♀								
22	♂	2.6 \pm 0.24	8.7 \pm 0.21	3.8 \pm 0.26	72.5 \pm 0.73	203.0 \pm —	45.7 \pm 0.97	25.3 \pm 0.31	361.5 \pm 1.09
	♀	3.4 \pm 0.13	7.2 \pm 0.54	4.0 \pm 0.33	75.5 \pm 0.85	203.0 \pm —	52.7 \pm 0.52	28.8 \pm 0.60	374.5 \pm 0.93
26	♂	1.9 \pm 0.18	5.0 \pm 0.13	2.6 \pm 0.15	78.6 \pm 0.44	203.0 \pm —	28.1 \pm 0.59	12.3 \pm 0.14	331.4 \pm 0.75
	♀	3.7 \pm 0.13	4.1 \pm 0.19	3.0 \pm 0.12	79.3 \pm 0.49	203.0 \pm —	29.6 \pm 0.43	13.2 \pm 0.24	335.8 \pm 0.63
29	♂	1.5 \pm 0.16	4.1 \pm 0.12	2.3 \pm 0.15	79.1 \pm 0.43	203.0 \pm —	22.7 \pm 0.38	10.3 \pm 0.25	322.8 \pm 0.53
	♀	3.7 \pm 0.13	3.6 \pm 0.19	2.3 \pm 0.13	80.6 \pm 0.50	203.0 \pm —	24.8 \pm 0.43	11.0 \pm 0.27	329.0 \pm 0.60
14:27	♂	2.3 \pm 0.15	6.7 \pm 0.14	3.3 \pm 0.22	75.3 \pm 0.56	203.0 \pm —	28.5 \pm 0.19	19.5 \pm 0.23	338.6 \pm 0.44
	♀	3.8 \pm 0.14	5.4 \pm 0.38	3.9 \pm 0.22	77.8 \pm 0.58	203.0 \pm —	30.6 \pm 0.25	20.9 \pm 0.45	345.5 \pm 0.74
Outside	♂	—	—	—	97.7 \pm 0.32	203.0 ^b \pm —	37.3 ^c \pm 0.19	17.8 \pm 0.18	358.1 \pm 0.42
	♀	—	—	—	97.9 \pm 0.47	203.0 ^b \pm —	39.9 ^c \pm 0.40	20.0 \pm 0.31	364.0 \pm 0.69

Def./Spin., period from first observation of individuals defecating (initiation of fifth instar) to time when first strands of silk are produced for cocooning by fifth instar individuals. Spin./Cool, period from first strands of silk produced by fifth instar individuals to wintering initiation. Cool/Inc., wintering period 13 October 1998–4 May 1999. Inc./Pupa, period from initiation of artificial incubation to pupal stage (4 May 1999).

^a No individuals completed development and emerged during period of investigation.

^b Based on estimated wintering period using maximum temperatures (28 October 1998) when they dropped consistently below 16°C.

^c Based on estimated end of wintering period using maximum temperatures (18 May 1999) when they increased consistently above 16°C.

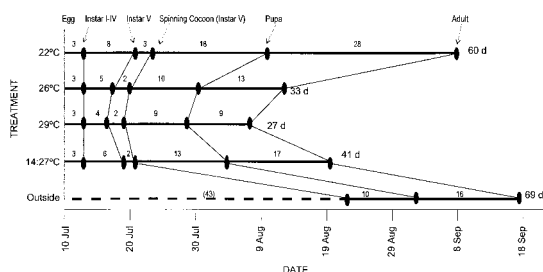


Fig. 1. Phenology of nondiapausing *M. rotundata* under various temperature regimes. Black dots represent mean times for each development stage (females and males combined). Small numbers indicate mean duration of each stage. Large numbers to the right indicate mean duration, in days, from egg to adult.

($F = 1,327.1$, $df = 4$, $P < 0.0001$) forms. There was a significant treatment by sex interaction in total developmental time for diapausing bees ($F = 9.6$, $df = 4$, $P < 0.0001$), but not for nondiapausing forms ($F = 0.24$, $df = 4$, $P > 0.92$).

Among the three constant temperatures under which bees were able to complete development and emerge, only bees at 22°C developed more slowly than those from the outside treatment. Both nondiapausing and diapausing bees from the treatment 14:27°C (mean, 22°C) developed faster from egg to adult (mean, 41 and 343 d for nondiapausing and diapausing forms, respectively) versus bees exposed to constant 22°C (mean, 60 d and 368 d for nondiapausing and diapausing forms, respectively) (Tables 1 and 2). A comparison of individual developmental stage durations revealed that the interval from pupa to adult showed the greatest reduction under the fluctuating temperature 14:27°C treatment versus the constant 22°C, for both nondiapausing (11 d reduction) and diapausing bees (7 d reduction) (Figs. 1 and 2).

The number of individuals in the population exhibiting nondiapause (Table 3) did not vary significantly among temperature treatments that we managed ($\chi^2 = 2.08$, $df = 4$, $P > 0.72$). Excluding the 18°C treatment, early developmental mortality, defined

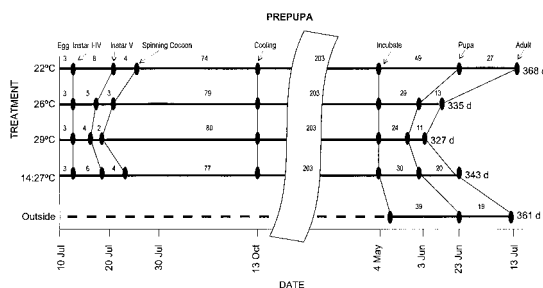


Fig. 2. Phenology of diapausing *M. rotundata* under various temperature regimes. Black dots represent mean times for each development stage (females and males combined). Small numbers indicate mean duration of each stage. Large numbers to the right indicate mean duration, in days, from egg to adult.

here as that portion of total mortality that occurred before the pupal stage in nondiapausing forms and before the wintering prepupal stage in diapausing forms, did not differ significantly among temperature treatments ($\chi^2 = 8.01$, $df = 4$, $P > 0.09$). Late developmental mortality for nondiapausing male and female bees combined differed significantly among temperature treatments ($\chi^2 = 9.34$, $df = 4$, $P = 0.05$) (Table 3). Late developmental mortality for diapausing males and females combined, which differed significantly among treatments ($\chi^2 = 62.57$, $df = 4$, $P < 0.001$), was highest in the constant 22°C and outside treatments and lowest in the constant 26°C and variable 14:27°C treatments (Table 3). Total percent mortality was highest in the constant 22°C and variable outside treatments, and lowest in the constant 26°C and variable 14:27°C (mean, 22°C) treatments.

There were temperature effects on adult emergence from two perspectives; that of the individual as well as the population (Tables 4 and 5). Most previous studies reported on the influence of various temperature regimes on the total time to emergence of individuals, as well as mean or median calendar date and range of days over which individuals emerged for the population and treatment (Richards and Whitfield 1988). With the use of X-ray technology, we were able to separate the postincubation prepupal period, the pupal period, and the adult to emergence period. Thus, when we report on postdiapause emergence times, we refer to the interval from the adult stage to emergence from the cocoon (Table 5; Fig. 2). By simply summing the three postincubation interval times that we report by treatment and sex (Table 2) or sexes combined (Fig. 2), a reasonable comparison can be made to postincubation development times reported previously.

Nondiapausing bees from all laboratory treatments 22, 26, 29, and 14:27°C emerged promptly, on average within 2–6 d after adulthood, during the autumn of 1998 (Table 4). Diapausing bees, which overwintered as prepupae and emerged during the early summer of 1999, on average required about twice that time to emerge (Table 5). For nondiapausing bees, there were detectable differences in emergence times among treatments ($F = 26.44$, $df = 4$, $P < 0.0001$), and males required less time at a given temperature to emerge ($F = 4.43$, $df = 1$, $P < 0.04$). For diapausing bees, there were also detectable differences in emergence times among treatments ($F = 207.06$, $df = 4$, $P < 0.0001$), but males required the same amount of time as females at a given temperature to emerge ($F = 3.12$, $df = 1$, $P > 0.08$). There was no evidence of a treatment by sex interaction in emergence times for either nondiapausing ($F = 0.10$, $df = 4$, $P > 0.98$) or diapausing bees ($F = 1.87$, $df = 4$, $P > 0.11$).

Nondiapausing bees emerged most rapidly under the 26 and 29°C treatments (Table 4). When reared under the 22°C treatment, nondiapausing bees required, on average, about two additional days to emerge compared with those held at 14:27°C (mean, 22°C; Table 4). Also, nondiapausing bees reared under outside conditions required about the same number of

Table 3. Sample sizes, percent nondiapausing, percent diapausing, and percent mortality for male and female *M. rotundata* reared under differing temperature regimes (%)

Treatment, °C	n	Early developmental mortality	Nondiapausing (1-yr) Forms	Diapausing (2-yr) Forms	Late developmental mortality		% total mortality
					Nondiapausing (1-yr) Forms	Diapausing (2-yr) Forms	
18 ^a	144						
22	144	23 (16)	41 (34)	80 (66)	11 (27)	23 (29)	40
26	145	12 (8)	50 (38)	83 (62)	6 (12)	2 (2)	14
29	146	17 (12)	53 (41)	77 (60)	10 (19)	6 (8)	21
14:27	142	10 (7)	53 (40)	79 (60)	9 (17)	4 (5)	16
Outside	365	32 (9)	119 (36)	214 (64)	10 (8)	85 (40)	35

^a No individuals completed development and emerged during period of investigation.

days as the only other variable temperature treatment, 14:27°C. The mean air temperature for this developmental period (first adult, 8 August 1998–last emerged bee, 7 September 1998) was 23°C (range, 19–26°C).

Diapausing bees emerged most rapidly under the 29°C treatment (Table 5). When reared under the 22°C treatment, diapausing bees required, on average, about two fewer days to emerge compared with those held at 14:27°C (Table 5), a reversal of what was observed in nondiapausing bees during the previous autumn. Also, when reared under outside conditions, diapausing bees required approximately four fewer days to emerge when compared with the only other variable temperature treatment, 14:27°C. Despite the fact that the mean air temperature for this developmental period (first adult 4 July 1999–last emerged bee 26 July 1999) was 22°C (range, 18–25°C), emergence times under the outside treatment were more similar to those from the constant 26°C, than they were to the variable 14:27°C (mean, 22°C).

At the population level, and as expected in this protandrous species, median emergence dates for males preceded those of females in all treatments, and in both nondiapausing and diapausing forms (Tables 4 and 5). Median calendar dates also revealed the same order of emergence for both nondiapausing (Table 4)

and diapausing (Table 5) forms, with populations from the constant 29°C treatment earliest followed sequentially by constant 26°C, variable outside, variable 14:27°C, and finally 22°C. Although both the constant 22°C and variable 14:27°C treatments maintained a mean temperature of 22°C, populations exposed to the variable temperature treatment emerged 3–4 wk earlier and emerged over a shorter period than at a constant 22°C.

Longevity of emerged bees, maintained upon emergence at 22°C without feeding, averaged 6 d overall for nondiapausing bees versus 5 d for diapausing bees (Tables 4 and 5). There were significant differences in longevity among nondiapausing bees that had been reared under the various temperature treatments ($F = 34.49$, $df = 4$, $P < 0.0001$), but not between sexes ($F = 1.00$, $df = 1$, $P > 0.31$). Likewise for diapausing bees, longevity was significantly influenced by rearing conditions ($F = 38.91$, $df = 4$, $P < 0.0001$), but males lived slightly longer than females ($F = 10.15$, $df = 1$, $P < 0.002$). There were no treatment by sex interactions in longevity for either nondiapausing ($F = 1.79$, $df = 4$, $P > 0.13$) or diapausing bees ($F = 2.03$, $df = 4$, $P > 0.09$).

Discussion

All empirical investigations concerned with the effects of differing temperature regimes on the development of *M. rotundata*, including the current study, implicitly assume that it is possible to select for thermal conditions that will ultimately yield rapid development, minimal mortality, short emergence intervals, and maximum longevity for this important agricultural pollinator. Our investigations differ from most other studies devoted to the temperature-dependent development of *M. rotundata* in that we report not only on stage-specific temperature effects on both nondiapausing as well as diapausing forms, but we also report on the effect of temperature on the development, emergence, and longevity observed over an entire generation of bees.

Studies of insect development generally show that development rates increase with increasing temperatures to some limit beyond which any gains in more rapid development are offset by increases in mortality rates (Ratte 1984). Although a lower developmental

Table 4. Median adult emergence dates, emergence times (in days at assigned treatment temperature), and longevity without feeding (in days at 22°C) for nondiapausing male and female *M. rotundata* (mean \pm SE)

Treatment, °C	Sex	Median emergence date for population	Range, d	Adult/ emergence	Longevity Without Feeding
18 ^a	♂				
	♀				
22	♂	17 Sept. 1998	18	5.3 \pm 0.37	2.9 \pm 0.23
	♀	18 Sept. 1998	20	5.8 \pm 0.47	3.4 \pm 0.40
26	♂	15 Aug. 1998	17	2.5 \pm 0.17	6.5 \pm 0.35
	♀	17 Aug. 1998	17	2.9 \pm 0.31	5.9 \pm 0.73
29	♂	10 Aug. 1998	5	1.9 \pm 0.18	6.0 \pm 0.41
	♀	11 Aug. 1998	13	2.2 \pm 0.28	4.3 \pm 0.69
14–27	♂	27 Aug. 1998	13	3.6 \pm 0.21	7.1 \pm 0.29
	♀	29 Aug. 1998	9	4.3 \pm 0.46	7.7 \pm 0.65
Outside	♂	19 Aug. 1998	25	4.1 \pm 0.29	7.9 \pm 0.27
	♀	25 Aug. 1998	7	4.7 \pm 0.30	7.6 \pm 0.34

^a No individuals completed development and emerged during period of investigation.

Table 5. Median adult emergence dates, emergence times (in days at assigned treatment temperature), and longevity without feeding (in days at 22°C) for diapausing male and female *M. rotundata* (mean \pm SE)

Treatment, °C	Sex	Median emergence date for population	Range, d	Adult/ emergence	Longevity without feeding
18 ^a	♂				
	♀				
22	♂	23 July 1999	21	8.7 \pm 0.28	2.2 \pm 0.19
	♀	02 Aug. 1999	11	8.4 \pm 0.52	2.8 \pm 0.35
26	♂	19 June 1999	19	6.0 \pm 0.17	5.3 \pm 0.27
	♀	23 June 1999	15	6.8 \pm 0.21	4.3 \pm 0.35
29	♂	08 June 1999	7	3.0 \pm 0.18	5.4 \pm 0.43
	♀	12 June 1999	10	3.3 \pm 0.21	4.7 \pm 0.44
14:27	♂	01 July 1999	8	10.0 \pm 0.18	5.6 \pm 0.24
	♀	06 July 1999	8	10.9 \pm 0.42	4.1 \pm 0.30
Outside	♂	16 July 1999	7	6.7 \pm 0.17	7.1 \pm 0.23
	♀	20 July 1999	5	6.6 \pm 0.31	6.2 \pm 0.38

^a No individuals completed development and emerged during period of investigation.

threshold of 15°C has been estimated for egg-fifth-instar *M. rotundata* (Whitfield and Richards 1992) and 15.7°C for prepupae (Richards and Whitfield 1988), our results show that it is not possible for either non-diapausing or diapausing forms to complete development and emerge at a constant 18°C. The results from previous investigations, where larval development of *M. rotundata* was monitored at constant temperatures of 15, 16, and 18°C, likewise show elevated mortality (Tepedino and Parker 1986, Whitfield and Richards 1992). Delayed development and emergence, as well as elevated mortality that we observed at a constant 22°C, indicate that this treatment is suboptimal as well. However, the overall poor performance of bees at a constant 22°C contrasts sharply with the excellent results from bees reared under the variable 14:27°C treatment, which resulted in a mean temperature of 22°C (Tables 1–5; Figs. 1 and 2). We have demonstrated the benefits of the variable 14:27°C (mean, 22°C) treatment elsewhere with the orchard pollinator *Osmia lignaria* Say (Bosch and Kemp 2000).

Constant 26 and 29°C temperatures and variable 14:27°C treatments produced rapid development, low overall mortality, rapid emergence, and vigorous adults. For diapausing bees under these three treatments, populations emerged 2–5 wk ahead of those held under seminatural outside conditions.

With the exception of Tepedino and Parker (1986), who reported in part on nondiapausing populations, our results compare well with previous investigations emphasizing the temperature-dependent development of diapausing *M. rotundata* (Bitner 1976, Taséi and Masure 1978, Whitfield and Richards 1992). The lack of significance that we observed among egg and larval rearing temperatures on the ultimate expression of nondiapausing forms (Table 3) is consistent with the results of Tepedino and Parker (1986). In an extensive investigation of immature *M. rotundata* development, Whitfield and Richards (1992) found that for early developmental stages, increasing temperatures

from 15 to 35°C resulted in faster development rates. However, in the last two development stages, during which most of the pollen/nectar provision is consumed, temperatures beyond 30°C resulted in a decrease in development rates, and a reduction in survival. Similarly, Undurraga (1978) found that eggs and young larvae reared at a constant 30°C demonstrated rapid development and survival rates in excess of 85% during most years. Of the various factors examined, the thermal history of immatures was the most important determinant of survival. Both Eves (1973) and Undurraga (1978) noted the importance of minimizing exposure of eggs and developing larvae to temperatures >30°C and prolonged exposure to temperatures >26°C. Given the heat retention characteristics of commercial-scale nesting materials (Peterson et al. 1994), ambient temperatures >26°C may be a major factor in explaining the high mortality of eggs and developing larvae observed during certain years and consistently in some geographic regions where alfalfa seed producers rely on the pollination services of *M. rotundata* (Kemp and Bosch 1998).

Only recently have scientists realized the importance of wintering conditions on the variability observed in temperature-dependent postdiapause prepupal development and adult emergence of *M. rotundata*. A series of investigations (Richards et al. 1987, Richards and Whitfield 1988) have demonstrated the need for standardization in both wintering duration and temperature to allow for comparisons among various investigations, as well as provide adequate conditions for diapausing bee populations, which in turn will help producers with the production of well-timed and vigorous pollinator populations for alfalfa seed each summer. Because we wintered bees at 4°C for 203 d in controlled treatments, our results compare more directly with Richards and Whitfield (1988) and Richards et al. (1987) than with related previous research involving a wide range of wintering conditions (Stephen and Osgood 1965, Kronic and Hinks 1972, Johansen and Eves 1973, Taséi and Masure 1978, Pankiw and Lieverse 1980, Undurraga and Stephen 1980, Rank and Goerzen 1982). The high late developmental mortality that we observed in the outside treatment (Table 3) indicates that ambient winter conditions in the Cache Valley of Northern Utah are suboptimal. The elevated late developmental mortality likewise observed in the 22°C treatment, despite adequate wintering conditions, further demonstrates the importance of avoiding suboptimal egg and larval rearing temperatures. In related research on the orchard pollinator *O. lignaria*, providing for adequate temperature regimes during development and/or wintering has permitted the establishment of late-flying (April–May) populations on early blooming (February) almond crops in California within one generation (unpublished data).

For both nondiapausing and diapausing forms, the most important result of elevated constant temperatures was the reduction in the duration of the prepupal and pupal stages. This was also true of the two variable temperature regimes despite the fact that mean tem-

perature for the variable 14:27°C was 22°C, and the variable outside treatment mean temperature was 18°C (for period during 1999, from 18 May when outside daily mean temperatures were consistently >16°C, through the last adult molt observed on 22 July) for the postdiapause development period. These results are similar to observations that we have made on the spring-emerging orchard pollinator *O. lignaria*, which exhibits shortened prepupal dormancy under fluctuating temperature regimes like those used in this study (Bosch and Kemp 2000).

Our results demonstrate the importance of differing temperature regimes on *M. rotundata* development, survival, and longevity over the entire life cycle. It is important to realize the connection between immature developmental and sufficient wintering conditions to postdiapause development, which has received much more attention in the literature. Further work is needed on the beneficial aspects of variable temperature regimes for immatures and postdiapause prepupal development of diapausing forms. Also needed is a logical schedule of events for prewintering and wintering *M. rotundata*, which will improve survival and result in well-timed and vigorous pollinator populations for alfalfa.

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